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The effect of chronic antidepressant administration on β-adrenoceptor function of the rat pineal

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1 The β-adrenoceptor agonist, isoprenaline (1.5–3.0 mg kg^{-1} intravenously), produced a dose-related increase in rat pineal melatonin content. This increase was prevented by pretreatment with the selective β_1-adrenoceptor antagonist, atenolol (2 mg kg^{-1}), but not by the β_2-adrenoceptor antagonist, butoxamine (2 mg kg^{-1}). The β_2-adrenoceptor agonist, terbutaline (5.0 mg kg^{-1}), produced a moderate increase in pineal melatonin content.

2 Repeated daily administration of desmethylimipramine (10 mg kg^{-1} for 10 days) and maprotiline (10 mg kg^{-1} for 10 days), antidepressants predominantly inhibiting noradrenaline (NA) uptake, reduced the isoprenaline-induced increase in pineal melatonin content. Amitriptyline (20 mg kg^{-1} for 14 days), a drug which inhibits both NA and 5-hydroxytryptamine (5-HT) uptake, had a similar effect. The β-adrenoceptor agonist, clenbuterol (5 mg kg^{-1} for 14 days), also attenuated the increase in pineal melatonin produced by isoprenaline.

3 In contrast, chronic administration of the selective 5-HT uptake inhibitor, fluoxetine (10 mg kg^{-1} for 10 days), or the antidepressants, iprindole and mianserin (both 20 mg kg^{-1} for 14 days), which do not inhibit monoamine uptake, failed to reduce the increase in pineal melatonin following isoprenaline. Repeated electroconvulsive shock was similarly without effect.

4 Ten hours after the final dose of desmethylimipramine (10 mg kg^{-1}) once daily for 10 days there was no change in the usual dark phase increase in pineal melatonin.

5 The data suggest that repeated administration of certain antidepressant drugs results in reduced pineal β-adrenoceptor sensitivity. However the lack of change in the dark phase increase in pineal melatonin following repeated desmethylimipramine, implies that the reduced ß-adrenoceptor sensitivity may be part of an adaptive process which maintains normal pineal function. Therefore the decrease in β-adrenoceptor number in the brain reported after chronic antidepressant administration may not be associated with a change in overall synaptic function.

Introduction

Recent research on the pharmacological effects of antidepressant drugs has focused on the adaptive changes produced by these compounds on central monoamine mechanisms. In particular it has been shown that many different antidepressant treatments, including repeated electroconvulsive shock (ECS), reduce β-adrenoceptor binding in certain regions of rat brain (Bergstrom & Kellar, 1979; Pandey, Heinze, Brown & Davis, 1979; Sellinger-Barnette, Mendels & Frazer, 1980). The reduction in β-adrenoceptor ligand binding is often, but not invariably (Mishra, Janowsky & Sulser, 1980), associated with a reduction in the responsiveness of noradrenaline-sensitive adenylate cyclase (Vetulani & Sulser, 1975; Wolfe, Harden, Sporn & Molinoff, 1978).

In order to demonstrate the consequences *in vivo* of longer term antidepressant administration on β-adrenoceptor sensitivity, we have measured the melatonin content of rat pineal gland since the production of melatonin is dependent on β-adrenoceptor stimulation (Axelrod, 1974). While the pineal gland may have direct neural connection with the brain (Dafny, 1980), its noradrenergic innervation is supplied by postganglionic sympathetic fibres whose cell bodies lie in the superior cervical ganglia (Moore, 1978). Experiments *in vitro* have demonstrated that the pineal β-adrenoceptors have characteristics of the β_1-subtype (Zatz, Kebabian, Romero, Lefkowitz & Axelrod, 1976; Bäckström, 1977) and in addition that the administration of desmethylimipramine (DMI) produces similar effects on pineal β-

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adrenoceptor binding and on cortical β-adrenoceptors (Moyer, Greenberg, Frazer, Brunswick, Mendels & Weiss, 1979).

To assess whether any particular pharmacological action of antidepressants could be linked with an effect on pineal β-adrenoceptors, we tested a number of antidepressant treatments which differ in their effect on monoamine uptake (see Iversen & Mackay, 1979). Of the tricyclic antidepressants tested, DMI inhibits the uptake of NA with a ten fold greater potency than that of 5-HT (Waldmeier, Greengrass, Baumann & Maitre, 1976), while amitriptyline is about equipotent in its inhibitory effect on 5-HT and NA uptake (Waldmeier *et al.*, 1976). The tetracyclic compound, maprotiline, potently inhibits NA uptake but has only a very weak effect on 5-HT uptake (Maitre, Greengrass, Jackel, Sedlacek & Delini-Stula, 1975). In contrast, fluoxetine inhibits 5-HT uptake, but has a negligible effect on uptake into catecholamine neurones (Fuller & Wong, 1977). The tricyclic antidepressant, iprindole, does not appear to block monoamine uptake to any great extent (Rosloff & Davis, 1974) and the tetracyclic antidepressant, mianserin, similarly has a weak effect on this process (Baumann & Maitre, 1977). In addition we tested the effect of the lipophilic β-adrenoceptor agonist, clenbuterol (Engelhardt, 1976) since β-adrenoceptor agonists are currently being evaluated as antidepressants.

Repeated administration of all these treatments except for flouoxetine (Peroutka & Snyder, 1980) and mianserin (Sellinger-Barnette *et al.*, 1980; Mishra *et al.*, 1980) reduce cortical β-adrenoceptor binding (Sellinger-Barnette *et al.*, 1980; Hall, Sällemark & Ross, 1980). The effect of maprotiline on brain β-adrenoceptor binding has not been described.

A preliminary account of some of this work has been presented to the British Pharmacological Society (Cowen & Fraser, 1981).

Methods

Animals

Male, Sprague-Dawley derived rats, 250–280 g at the time of testing, were used. They were kept in a 12 h light: 12 h dark schedule (lights on at 08 h 00 min–20 h 00 min) in a constant temperature (20°C ± 1°C) and fed *ad libitum* on a diet of modified 41B pellets and tap water.

Pineal response test

Isoprenaline was administered intravenously (i.v.) in the light phase at 11 h 00 min and animals killed 3 h later. The pineals were then quickly dissected out and frozen at − 20°C. For dark phase experiments rats were killed under a dim red light and the pineals removed and frozen. Because of the well-known diurnal variation in pineal β-adrenoceptor binding and cyclic AMP response (Romero, Zatz, Kebabian & Axelrod, 1975) and the seasonal variations in pineal activity and melatonin production (Arendt, Wirz-Justice, Bradtke & Kornemark, 1979), a control group of animals was always tested with isoprenaline at the same time as the treatment group.

Assay of melatonin

Pineals were homogenized in sodium phosphate buffer (100 mM, pH 7.1) and the melatonin content determined by a previously described radioimmunoassay (Arendt, Paunier & Sizonenko, 1975; Arendt & Wilkinson, 1979). Pineals were assayed in duplicate. Sensitivity of the assay was 8 pg/tube, while intra- and interassay variation were 10 and 13% respectively. Experimental and control pineals were always assayed together.

β-Adrenoceptor binding

Animals were killed at 14 h 00 min and pineals removed and frozen. Glands from three animals from similar treatment groups were pooled, homogenized in 6 ml Tris buffer (50 mM, pH 7.8) and centrifuged at 20,000 *g*. After washing, resuspension and recentrifugation, the final pellet was resuspended in 700 μl 50 mM Tris buffer (pH 7.8). Four aliquots of 130 μl were taken for radioligand binding while a further 130 μl aliquot was used for protein determination (Lowry, Rosebrough, Farr & Randall, 1951).

Samples were incubated in 0.75 mM [^{3}H]-dihydroalprenolol (DHA) (26°C, 40 min), filtered over Whatman GF/B glass fibre filters, washed with 15 ml of the Tris buffer and then taken for tritium counting. Non-specific binding was taken as that found in tissues pre-incubated with 34 μM (±)-propranolol 26°C; 30 min). Specific binding of [3H]-DHA was calculated as total minus non-specific bound and represented approximately 50% of total bound. The total incubation value was 1.0 ml and all samples were studied in duplicate.

Drugs

Isoprenaline was administered as the commercial preparation ('Suscardia', Pharmax). Isoprenaline vehicle was made up as follows: ascorbic acid 2 mg, disodium edetate (EDTA) 0.4 mg, hydrochloric acid 0.5 μl and distilled water to a volume of 2 ml and final pH of 3. Terbutaline was also administered as the commercial preparation ('Bricanyl', Astra).

The following drugs were kindly donated by the Companies concerned: amitriptyline (Roche), atenolol (ICI Pharmaceuticals), butoxamine (Burroughs Wellcome), clenbuterol (Boehringer-Ingelheim), DMI and maprotiline (Ciba-Geigy), fluoxetine (Eli Lilly), iprindole (Wyeth Laboratories) and mianserin (Organon).

All these drugs were dissolved in saline except for maprotiline, which was dissolved in distilled water.

Administration of drugs and electroconvulsive shock (ECS)

Isoprenaline and terbutaline were administered i.v. via a tail vein. All other drugs were given intraperitoneally (i.p.). Chronically administered drugs were injected at a dose of 10 mg kg^{-1} either once daily at 14 h 00 min (DMI, maprotiline and fluoxetine) or twice daily at 09 h 00 min and 18 h 00 min (amitriptyline, mianserin, iprindole). Clenbuterol was injected once daily at a dose of 5.0 mg kg^{-1}. Isoprenaline testing was carried out as described on the day following the final injection.

ECS (125 V, 50 Hz sinusoidal for 1 s) was given via earclip electrodes once daily at 14 h 00 min for 10 days. A grand mal seizure was always observed. Control animals were similarly handled, electrodes placed but no current passed ('handled'). Isoprenaline testing was carried out the next day.

Statistics

Differences between control and experimental groups of animals were analysed by Student's unpaired *t* test (two-tailed).

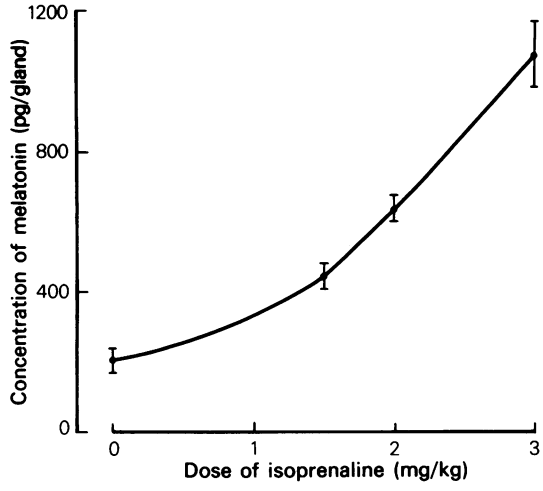

Figure 1 The effect of isoprenaline on melatonin content of the pineal gland. Isoprenaline was injected i.v. and pineals removed 3 h later. Each point shows the mean melatonin content of 12 pineals in pg/gland; vertical lines indicate s.e.mean.

Results

Effect of isoprenaline on the synthesis of melatonin by the pineal gland

An i.v. injection of 0.9% w/v NaCl solution (saline) or vehicle (see Methods) at 11 h 00 min had no effect on the concentration of melatonin in the pineal. Isoprenaline produced a dose-related increase in pineal melatonin content 3 h later (Figure 1).

Table 1 Effect of acute administration of β-adrenoceptor agonists and antagonists on pineal melatonin content

Treatment		*Melatonin content pineal* (pg/gland)
Nil		158 ± 20 (6)
Saline		155 ± 18 (7)
Vehicle		150 ± 17 (5)
Isoprenaline + saline	(2.0 mg kg^{-1})	625 ± 46 (6)
Isoprenaline + atenolol	(2.0 mg kg^{-1}) (2.0 mg kg^{-1})	223 ± 22 (6)*
Isoprenaline + butoxamine	(2.0 mg kg^{-1}) (2.0 mg kg^{-1})	609 ± 74 (6)
Terbutaline	(2.0 mg kg^{-1})	163 ± 27 (6)
	(5.0 mg kg^{-1})	312 ± 19 (5)**

Saline, vehicle, isoprenaline and terbutaline were given i.v. Saline, butoxamine and atenolol were given i.p. 15 min before isoprenaline. Pineals were removed 3 h later. Figures in parentheses are number of pineals assayed. Values are mean ± s.e.mean.

*$P < 0.001$ compared to isoprenaline + saline; **$P < 0.001$ compared to vehicle.

Characterization of the pineal β-adrenoceptor

Pretreatment of animals with atenolol ($2\,mg\,kg^{-1}$ i.p.) 15 min before isoprenaline ($2\,mg\,kg^{-1}$ i.v.) prevented the increase in pineal melatonin content (Table 1). Butoxamine ($2\,mg\,kg^{-1}$) did not block the effect of isoprenaline (Table 1). Administration of the β_2-adrenoceptor agonist, terbutaline, at a dose of $2\,mg\,kg^{-1}$ i.v. failed to alter pineal melatonin content but a dose of $5\,mg\,kg^{-1}$ i.v. did increase pineal melatonin (Table 1).

Effect of repeated administration of antidepressant drugs, a β-adrenoceptor agonist and ECS on the isoprenaline-induced increase in pineal melatonin

In animals given a saline injection once daily for 10 days, isoprenaline ($1.5\,mg\,kg^{-1}$) produced an increase in pineal melatonin concentration 24 h after the last injection (Table 2).

When rats were given saline once daily for 9 days followed by DMI ($10\,mg\,kg^{-1}$) on day 10, there was no significant alteration in the isoprenaline stimulation of pineal melatonin content 24 h later. However, 24 h after the final dose of DMI ($10\,mg\,kg^{-1}$) given once daily for 10 days, the isoprenaline-induced melatonin rise was abolished. In addition, the basal melatonin concentration was decreased (Table 2). Another relatively selective NA uptake inhibitor, maprotiline ($10\,mg\,kg^{-1}$ for 10 days), also reduced both the basal melatonin level and the increase in melatonin following isoprenaline (Table 2). Amitriptyline ($10\,mg\,kg^{-1}$ twice daily for 14 days) which inhibits both NA and 5-HT uptake did not decrease the basal melatonin concentration but did produce a moderate reduction in the pineal response to isoprenaline (Table 2).

In contrast, 14 days' treatment with the antidepressants, mianserin and iprindole (both $10\,mg\,kg^{-1}$ twice daily), did not reduce either the basal melatonin concentration or the response to isoprenaline (Table 2). Neither of these drugs inhibits monoamine uptake (see Discussion).

When the specific 5-HT uptake inhibitor, fluoxetine ($10\,mg\,kg^{-1}$) was administered for 10 days there was no effect on basal melatonin concentration or the isoprenaline-induced melatonin increase. However, treatment with the β-adrenoceptor agonist, clenbuterol ($5\,mg\,kg^{-1}$), for 14 days, whilst not

Table 2 Effect of repeated administration of antidepressant drugs, selective monoamine uptake inhibitors, β-adrenoceptor agonists and ECS on the isoprenaline-induced increase in pineal melatonin

Treatment	*Duration (days)*	*Vehicle*	*Isoprenaline*
Antidepressants predominantly affecting NA uptake			
Saline	10	250 ± 11 (6)	440 ± 55 (7)
DMI	1	239 ± 12 (7)	366 ± 50 (6)
DMI	10	177 ± 16 (6)**	204 ± 21 (7)**
Saline	10	185 ± 13 (6)	436 ± 60 (5)
Maprotiline	10	139 ± 15 (7)†	263 ± 18 (7)††
Antidepressant affecting both NA and 5-HT uptake			
Saline	14	178 ± 22 (8)	654 ± 50 (8)
Amitriptyline	14	188 ± 25 (7)	518 ± 23 (7)†
Antidepressants not affecting monoamine uptake			
Saline	14	141 ± 17 (7)	510 ± 55 (7)
Mianserin	14	162 ± 18 (7)	535 ± 61 (6)
Iprindole	14	167 ± 7 (6)	480 ± 29 (6)
Selective 5-HT uptake inhibitor			
Saline	10	194 ± 19 (7)	466 ± 92 (7)
Fluoxetine	10	166 ± 34 (7)	444 ± 40 (7)
β-adrenoceptor agonist			
Saline	14	185 ± 13 (6)	433 ± 60 (9)
Clenbuterol	14	152 ± 16 (7)	221 ± 22 (7)*
Electroconvulsive shock			
Handled	10	197 ± 28 (5)	443 ± 37 (6)
ECS	10	195 ± 27 (5)	414 ± 36 (7)

Chronic treatments were administered as described in Methods. Isoprenaline ($1.5\,mg\,kg^{-1}$) or vehicle were given i.v. and pineals removed 3 h later. Values are mean ± s.e.mean pineal melatonin content in pg/gland.
**$P < 0.005$; *$P < 0.01$; ††$P < 0.025$; †$P < 0.05$ compared to appropriate saline control

significantly reducing the basal melatonin content, did attenuate the pineal response to isoprenaline (Table 2).

Finally, repeated electroconvulsive shock was found to have no effect on either the basal melatonin content or the isoprenaline-induced rise.

Effect of repeated desmethylimipramine administration on the night-time rise in pineal melatonin concentration

At midnight, 10 h after the last of 10 once daily saline injections, animals showed the usual marked nocturnal rise in pineal melatonin content (Arendt, Ho, Laud, Marston, Nohria, Smith & Symons, 1981). This rise was not significantly altered by chronic treatment with DMI (10 mg kg^{-1} daily for 10 days) (saline, 1621 ± 138 pg/gland, $n = 7$; DMI, 1407 ± 151 pg/gland, $n = 7$).

Effect of repeated ECS or desmethylimipramine on [^{3}H]-dihydroalprenolol binding

Repeated ECS did not alter the specific binding of [^{3}H]-DHA in the pineal gland 24 h after the last treatment. In contrast 24 h after repeated DMI there was a 20% decrease in [^{3}H]-DHA binding but this was not statistically significant (Table 3).

Discussion

The pineal enzyme, serotonin (5-HT) N-acetyltransferase (SNAT), catalyses a critical regulatory step in the formation of melatonin (Axelrod, 1974). SNAT is synthesized in a dose-dependent manner following administration of the β-adrenoceptor agonist, isoprenaline (Axelrod, 1974). Our results show that the increase in pineal melatonin following isoprenaline is also dose-dependent and that the increase is prevented by the β_1-adrenoceptor antagonist, atenolol, but not by the β_2-adrenoceptor antagonist, butoxamine. The β_2-adrenoceptor agonist, terbutaline, at a dose of 5 mg kg^{-1}, did produce a modest increase in pineal melatonin content. This finding is consistent with previous experiments *in vitro* which demonstrated that while terbutaline has agonist activity at pineal β-adrenoceptors, its affinity is less than that of a selective β_1-adrenoceptor agonist (Backström, 1977). Pineal β-adrenoceptors have characteristics *in vitro* of the β_1-subtype (Zatz *et al.*, 1976); however, it appears, in common with certain other β_1-adrenoceptors (Lecler, Rouot, Schwartz & Vetty, 1981), that they respond to β_2-adrenoceptor agonists if a sufficient dose is administered. Overall, therefore, our observations are in accord with the *in vitro* data.

Atenolol crosses the blood-brain barrier poorly (Day, Hemsworth & Street, 1977) and its blockade of the isoprenaline-induced increase in melatonin content is consistent with evidence suggesting that the pineal gland lies outside the blood-brain barrier (Arendt *et al.*, 1981). The effectiveness of isoprenaline in increasing melatonin is also in keeping with this interpretation. It has been reported that various stresses, including saline injection, can increase pineal melatonin levels (Lynch, Wang & Wurtman, 1973; Lynch, Eng & Wurtman, 1973), but in our studies administration of either i.v. saline or vehicle was without such effect.

Many antidepressant treatments, when administered for periods similar to those required to produce a clinical antidepressant effect, cause a reduction in the number of β-adrenoceptor binding sites (see Introduction). This change in general correlates with the ability of the drugs to inhibit NA uptake. Down-regulation in postsynaptic β-adrenoceptor number is thought to be an adaptive change to the increase in synaptic cleft concentration of noradrenaline (see Green & Nutt, 1982). However, inhibition of NA uptake is not the only mechanism involved in β-adrenoceptor down-regulation. Iprindole, a tricyclic antidepressant, does not appear to block monoamine uptake (Rosloff & Davis, 1974; Iversen & Mackay, 1979); nevertheless it reduces cortical β-

Table 3 Pineal [^{3}H]-dihydroalprenolol ([^{3}H]-DHA) binding following ECS and desmethylimipramine (DMI)

Treatment	*[^{3}H]-DHA binding* (pmol/g protein)
Handled × 10	50.5 ± 6.2 (4)
ECS × 10	49.2 ± 8.4 (5)
Saline × 10	54.3 ± 10.6 (4)
DMI × 10	44.3 ± 15.3 (4)

ECS and DMI were administered once daily for 10 days (× 10). Pineals were removed 24 h after the last treatment and binding performed as described in Methods. Figures in parentheses are number of binding samples. Each sample consisted of three pineals. Values are mean ± s.e.mean.

adrenoceptor binding (Wolfe *et al.*, 1978). In addition, there is evidence that the β-adrenoceptor agonist, clenbuterol, also down-regulates central β-adrenoceptors (Hall, *et al.*, 1980) and this is presumably a consequence of chronic agonist stimulation.

The synthesis of melatonin by the pineal gland in response to isoprenaline challenge provides a functional index of β-adrenoceptor sensitivity (Axelrod, 1974) and we have used this model to investigate the effects of various antidepressants on β-adrenoceptor function. Previous reports have indicated that repeated DMI administration reduces both pineal β-adrenoceptor binding and the maximum cyclic AMP response to noradrenaline and isoprenaline (Moyer *et al.*, 1979). Our results show that following 10 days DMI administration there is an attenuation of the isoprenaline-induced increase in pineal melatonin content, suggesting a decreased sensitivity of the pineal to β-adrenoceptor stimulation. Similarly, the reduction in pineal isoprenaline response following repeated amitriptyline and clenbuterol, is consistent with the reported effects of these drugs on cortical β-adrenoceptor binding (Sellinger-Barnette *et al.*, 1980; Hall *et al.*, 1980) although there is no information about their effect on pineal β-adrenoceptor binding. We have been unable to find data on the effect of the tetracyclic antidepressant, maprotiline on cortical β-adrenoceptor binding. However, a reduction in receptor density following chronic administration of this drug might be expected since it is a potent noradrenaline uptake inhibitor (Iversen & Mackay, 1979), and as shown here, reduces the pineal response to isoprenaline.

In the animals treated with DMI and maprotiline, interpretation of the reduced isoprenaline response is complicated by the lower basal levels of melatonin in the animals given i.v. vehicle. However, the percentage increase in pineal melatonin in the maprotiline-treated animals following isoprenaline is less than that of controls and in the case of DMI there is no significant increase at all following isoprenaline. This suggests that as well as lowering basal levels of melatonin, repeated DMI and maprotiline do reduce the pineal β-adrenoceptor response to isoprenaline.

However, chronic DMI did not significantly alter the usual dark phase increase in pineal melatonin, suggesting that the decrease in β-adrenoceptor sensitivity may be part of an adaptive response which maintains normal pineal function during repeated DMI administration. In contrast to certain electrophysiological investigations (Huang, Maas & Hu, 1980), behavioural studies have suggested that a reduction in noradrenergic function is not seen during a course of DMI treatment, but rather, during the withdrawal period (Willner & Montgomery, 1981; Willner, Montgomery & Bird, 1981). It is possible, therefore, that the lowered basal levels of pineal melatonin 24 h after the final injection of DMI and maprotiline also represent a withdrawal phenomenon. Other explanations for the failure of chronic DMI to alter night-time pineal activity are also possible. The increase in presynaptic NA release and in pineal β-adrenoceptor stimulation at this time might be too great for a relatively small decrease in receptor sensitivity to be detected. However, dose-response curves might well clarify this point. Alternatively, the influence of DMI on brain circadian rhythms may have obscured a concomitant change in pineal receptor sensitivity (Kafka, Wirz-Justice & Naber, 1981). If, however, the β-adrenoceptor down-regulation in the pineal following antidepressants does not alter the overall function of pineal NA synapses then it suggests that care should be taken in attributing importance to the process of β-adrenoceptor down-regulation as part of the therapeutic mechanism of antidepressant drugs (e.g. Sulser, 1979).

Mianserin has little effect on monoamine uptake and although it has been reported to block α_2-adrenoceptors when given acutely, this effect is not seen following longer term administration (see Green & Nutt, 1982). Repeated administration of mianserin does not produce a reduction in cortical β-adrenoceptor binding, even after long-term treatment at high dose (Sellinger-Barnette *et al.*, 1980; Mishra *et al.*, 1980).

The selective 5-HT inhibitor, fluoxetine, similarly does not change cortical β-adrenoceptor binding (Peroutka & Snyder, 1980). Chronic administration of these drugs did not change the pineal response to isoprenaline which is in accord with their lack of effect on cortical β-adrenoceptor binding. In contrast, it has been reported that repeated ECS reduces cortical β-adrenoceptor binding (Pandey *et al.*, 1979) but this treatment failed to alter the pineal response to isoprenaline in the present experiments. However, the effect of repeated ECS on brain β-adrenoceptor binding shows regional specificity (Stanford & Nutt, 1982) and our binding data suggest that repeated ECS does not alter pineal β-adrenoceptor density. The results of the binding data following repeated DMI were less conclusive, although the percentage decrease in β-adrenoceptor binding in the pineal was similar to that seen in rat cortex following the same DMI regimen (Stanford, Nutt & Cowen, 1983). A better approach would have been a full Scatchard analysis over a range of radioligand concentrations. However, this investigation would have needed a very large number of animals since even the present single point binding determination required pooling of glands.

Iprindole has been reported to reduce cortical β-adrenoceptor binding (Wolfe *et al.*, 1978). Its failure to alter pineal β-adrenoceptor function, therefore, may reflect differences in the properties of

pineal and cortical β-adrenoceptor synapses. Alternatively, since the reduction in β-adrenoceptor density following iprindole is usually somewhat less than that produced by DMI and amitriptyline (Sellinger-Barnette *et al.*, 1980; Peroutka & Snyder, 1980), it may be that any reduction in pineal β-adrenoceptor density caused by iprindole is insufficient to result in a decreased functional response to isoprenaline.

Our findings suggest that repeated administration of certain antidepressant treatments results in reduced pineal β-adrenoceptor responses. In the rat, plasma melatonin levels correlate closely with pineal melatonin synthesis (Wilkinson, Arendt, Bradtke & Ziegler, 1977) and it therefore seems possible that in man, measurement of plasma melatonin may provide a means of assessing alterations in β-adrenoceptor function during tricyclic antidepressant treatment.

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